

**IRON CHELATORS OF THE PYRIDOXAL-BASED CLASS. PART 7.¹
THE SYNTHESIS AND SINGLE CRYSTAL STRUCTURE OF 1-(*N*-ETHOXYCARBONYLMETHYLPYRIDOXYLEDENIUM)-2-(PYRIMIDYL)HYDRAZINE SALTS[‡]**

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Abstract - The syntheses of 1-pyridoxylidene-2-(2'-pyrimidyl)hydrazine (**1**), 1-(*N*-methylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine iodide (**2**), and 1-(*N*-ethoxycarbonylmethylpyridoxylidenium)-2-(pyrimidyl)hydrazine bromide (**5a**), and 1-(*N*-ethoxycarbonylmethylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine perchlorate (**5b**) are described. The single-crystal structure of **5b** was determined from three-dimensional X-Ray data. Compound (**5b**), C₁₆H₂₂N₅O₈Cl, crystallizes in the space group **P2₁/c** with **Z = 4** and the following cell dimensions : *a* = 12.363 (3) Å, *b* = 17.168(5) Å, *c* = 9.657(3) Å. The X-Ray data confirm that **5b** crystallizes in the *di-polar form*, as a planar 20-membered ring dimer {preferred motif of **R**²(20)}. All the three proton-donors (O¹-H, O²-H, and N³-H), and only three (N², N⁵, O³) of the five available proton-acceptors **5b**, are utilized in hydrogen-bonding.

INTRODUCTION

Pyridoxal isonicotinoyl hydrazone (PIH),¹ and its analogs: 1-pyridoxylidenium-2-(2'-pyridyl)hydrazine (PPH),² 1-(*N*-methylpyridoxylidenium)-2-(2'-pyridyl)hydrazine iodide (MPH),² and 1-(*N*-ethoxycarbonylmethylpyridoxylidenium)-2-(2'-pyridyl)hydrazine (EPH),³ represent a new class of biologically active lipophilic chelator that are endowed with a high affinity for both iron(II), and Fe(III) and are orally active, therapeutically safe,⁴ and of low cost. Their biological profiles comprise : (i) effective removal of toxic iron accumulations from the parenchymal iron-pool⁵ and from reticulocytes;⁶ (ii) parasitocidal activity against drug-resistant species of *Plasmodium falciparum* (the main causative agent of human

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‡ Dedicated to Dr. Bernhard Witkop on the occasion of his 80th birthday in appreciation for his excellence in Science and dedication to human values

malaria);⁷ and (iii) inhibition of the metalloenzyme ribonucleotide diphosphate reductase (essential to DNA synthesis).⁸ The evidence now at hand indicates that, depending on the nature of the hydrazone bridge between the pyridoxal and the pyridinic rings, the new class of chelators comprise two sub-classes, I and II. The first, of which PIH is a representative, contains a carbonyl group at the hydrazone-bridge (acylhydrazone). The second, represented by PPH, lacks a carbonyl group in its hydrazone bridge. Electronically, the hydrazone-bridge confers upon the chelator the ability to transmit conjugative effects between the two heterocyclic rings.⁹

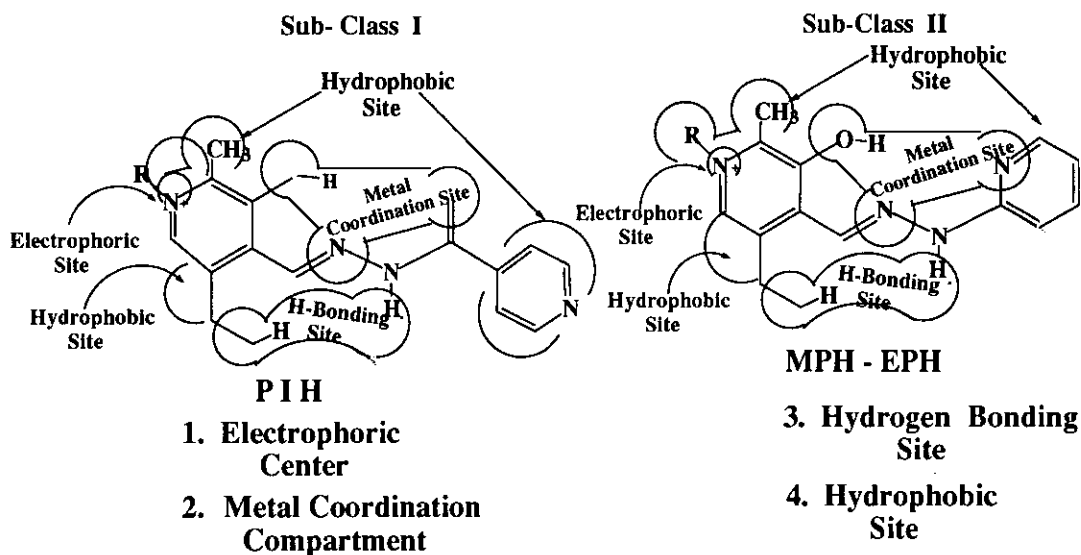
Studies indicated that the annular heteroatoms in PIH, PPH, MPH, and EPH, affect both the chemical and the biological properties of the ligands. The chelators appear to contain four distinctly different domains (see Scheme 1). The first domain comprises a *metal coordination compartment* containing three heteroatoms: O¹, N², and/or O³, or N⁴. It confers tridentate functionality on the chelator.¹⁰ The avidity of the chelator for the Fe²⁺-Fe³⁺ ions is conceivably influenced by the electron density around this domain (substitution effects). The second domain is an *electrophoric center*, and is located at the pyridoxal ring-N¹. At the demand of electron-deficient species (metal ions) it functions as a chemical pawnbroker, storing electrons for chemical purposes until new molecules arrive to claim them (thus behaving as an electron-sink).¹¹ The third domain comprises the *hydrogen-bonding sites*, including¹⁻³: O¹, O², N¹, N², N³, and N⁴. The fourth one comprises the *hydrophobic domain*, including the aromatic and the aliphatic hydrocarbon zones. It endows the chelator with a degree of lipophilicity thereby facilitates trans-membrane passage into the cell.

We previously showed¹² that protonation at the pyridoxal annular heteroatom lowers the potential energy of the ligand. Energetically, the ligands prefer a *trans* geometry around the hydrazone C=N double-bond. It has been found that quaternization of the annular heteroatoms in PIH (sub-class I) renders the salt liable to disproportionation under the influence of Fe²⁺ ions. The dismutation leads to the formation of the respective pyridoxal azine, and di-isonicotinoyl products.¹³ Similar quaternization of PPH (sub-class II) does not render the chelator liable to dismutation, although it apparently enhances the propensity of the deprotonated pyridoxal ring to undergo lactam/lactim tautomerization.^{2b} At the same time, it lowers its propensity to enter into intermolecular H-contacts.¹⁻³ Solution chemistry³ has indicated that, depending on pH and substitution, all pyridoxal-based chelators exist as mixtures of structural forms with variable potential energies. Evidently, these forms play a pivotal role in biological processes.^{3,11}

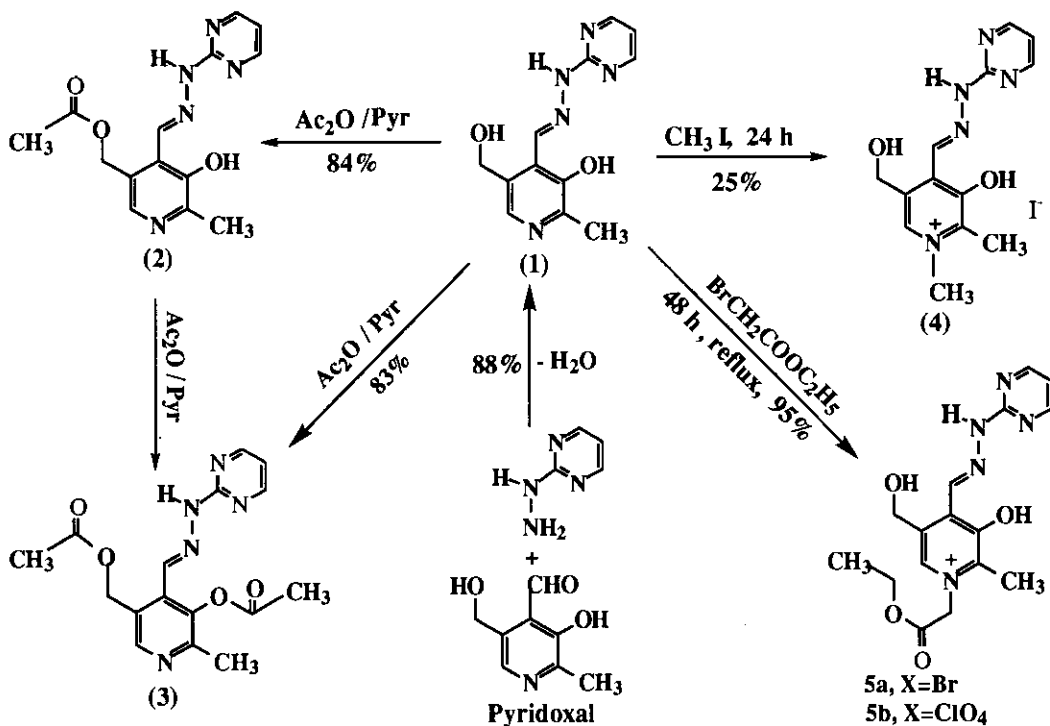
The substitution effects of iron mobilization are well documented. Thus, iron excretion in hypertransfused rats labelled with ⁵⁹Fe was found to *increase* in the order: PIH < diMe-PIH < PPH < MPH < DFO (desferrioxamine B, desferal, DF) < EPH.^{4a} Similarly, the antimalarial activity followed the order: PIH << DFO << PPH < MPH < EPH.¹⁵ These data suggested that the biological performance of the above chelators could be improved by replacing the pyridinic ring in PPH-EPH with the less basic pyrimidinic ring (pKa = 2.3).¹⁶ This led to the synthesis of a new series of analogs: 1-pyridoxylidene-2-(2'-pyrimidyl)hydrazine [PPmH](1), 1-(*N*-methylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine iodide [MPmH] (4), and 1-(*N*-ethoxycarbonylmethylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine bromide [EPmH](5), using conventional methods. The latter were screened for oral-removal of iron surpluses from the body (in rat models), and for schizonticidal (antimalarial) activity. As postulated, the pyrimidinic analog (EPmH) proved to be superior as an iron mobilizer, in comparison with its pyridinic counterpart (EPH), according to the following order: MPH < MPmH < EPH < EPmH.^{4a}

Scheme 1

The Four Domains of Pyridoxal-Based Chelators



Scheme 2



To shed additional light on the mechanisms underlying the biological properties of EPmH (5), we performed X-Ray analysis of crystalline EPmH-perchlorate (5a), with a view to uncovering new patterns in the mode of hydrogen bonding.

EXPERIMENTAL

Materials. 2-Hydrazinopyrimidine (mp 110-111°C)¹⁸ was prepared as described.^{5b}

IR were measured with a Perkin-Elmer Model 457 Grating infrared spectrophotometer. ¹H and ¹³C *NMR* spectra were taken on a Varian VXR 300 Spectrometer, with TMS as an internal standard and DMSO-d₆ as solvent. *UV* were run on a Kontron Uvikon 810 spectrophotometer. *MS* were recorded with a LKB 90 spectrometer. *Elementary analyses* were performed by the Microanalytical Unit of the Hebrew University, Dept. of Organic Chemistry, Givat Ram campus, Jerusalem.

1-Pyridoxylidene-2-(2'-pyrimidyl)hydrazine (1). To a solution of pyridoxal hydrochloride (2.0 g, 10 mmol) in 10 mL of water a solution of 2-hydrazinopyrimidine (1.32 g, 12 mmol) in ethanol (15 mL) was added dropwise with stirring at rt followed by the addition of 5 mL of 0.2 NaOH. After 5 to 6 h of stirring at rt, a yellow precipitate was collected (2.29 g, 88%), and recrystallized from ethanol, mp 298°C. The free base showed *UV* absorption bands (MeOH, nm) at: 342.5 (log ε 4.11), 339.8 (4.10), 307.4 (4.21), 323.2 (4.12), 208.5 (4.05). ¹H *NMR* (220 MHz, DMSO-d₆): δ 12.56 (1H), 12.02 (1H), 8.78 (1H, s), 8.66 (2H, s), 8.01 (1H, s), 7.74 (1H), 5.44 (1H, s), 4.68 (2H, s, CH₂), 2.50 (3H, s, CH₃). *MS* (*EI*) at *m/z* (relative intensity): 259 (M⁺, 60), 165 {[C₈H₉N₂O₂]⁺, 85}, 150 {[C₈H₈NO₂]⁺, 95}, 110 {[C₆H₈NO]⁺, 100%}, 68 {[C₃H₄N₂]⁺, 80}. *Anal.* Calcd for C₁₂H₁₃N₃O₂: C, 55.59; H, 5.02; N, 27.02. Found: C, 55.56; H, 5.03; N, 26.76. **The Hydrochloride** melted at mp > 350°C. *UV* (MeOH, c. 5 × 10⁻⁶ M) λ_{max} 361 nm (log ε 4.22), 321.2 (4.12), 236.1 (4.16), 211.8 (4.08), 205.5 (4.03). ¹H *NMR* (300 MHz, DMSO-d₆): δ 12.37 (1H, s), 11.13 (1H, s), 8.80 (2H, s), 8.65 (1H, s), 7.84 (2H, d, J = 1.3 Hz), 7.45 (1H, m), 6.91 (1H, d, J = 1.5 Hz), 3.36 (3H, d, J = 1 Hz). *Anal.* Calcd for C₁₂H₁₄N₃O₂Cl: C, 48.71; H, 4.73; N, 23.67. Found: C, 48.76; H, 4.49; N, 23.63.

1-(Pyridoxylidene-O²-acetate)-2-(2'-pyrimidyl)hydrazine (2). (a) *Semihydrate.* On reacting 1-pyridoxylidene-2-(2'-pyrimidyl)hydrazine (1) (0.5 g, 2 mmol) with acetic anhydride (15.6 mL, 2 mmol) in the presence of dry pyridine (9.4 mL) for 2 h at rt, a semi-hydrated mono-O²-acetate of 2 (0.5 g, 86%) was obtained. Recrystallization from MeOH yielded colorless crystals, mp 249°C. *Anal.* Calcd for C₁₄H₁₅N₃O₃·0.5H₂O: C, 54.19; H, 5.16; N, 22.58. Found: C, 54.40; H, 4.76; N, 22.49. (b) **Water-free mono-O²-acetate (2).** On drying the above semi-hydrated product over P₂O₅ its mp rose to 268°C, giving the correct elemental analysis for C₁₄H₁₅N₃O₃. *Anal.* Calcd: C, 55.81; H, 4.98; N, 23.21. Found: C, 55.46; H, 5.03; N, 22.78. *IR* (KBr, cm⁻¹): 3210, 3160, 3120, 3005, 2990, 2940, 2440, 2280, 1735, 1570, 1540, 1530, 1480, 1440, 1400, 1360, 1275, 1190, 1020, 1005, 965, 930, 905, 660, 790, 775, 715. ¹H *NMR* (300 MHz, DMSO-d₆): δ 8.59 (1H, s), 8.58 (1H, s), 8.50 (1H, s), 8.00 (1H, s), 7.00 (1H, t, J = 0.6 Hz), 5.17 (2H, s), 3.32 (4H, s), 2.43 (3H, s, CH₃), 2.07 (3H, s, CH₃). *MS* (*EI*) *m/z* (relative intensity): 301 {M⁺, base peak, 100%}, 283 {M-H₂O, 30}, 258 {M-CH₃CO, 40}, 242 {M-CH₃COO, 90}, 241 {M-CH₃COOH, 98}, 96 {C₄H₆N₃]⁺, 100%}.

1-(Pyridoxylidene-O¹,O²-diacetate)-2-(2'-pyrimidyl)hydrazine (3). 1-Pyridoxylidene-2-(2'-pyrimidyl)hydrazine (1) (0.5 g, 2 mmol) was reacted with acetic anhydride (22 mL, 3 mmol) in the presence of dry pyridine (20 mL) at rt for 12 h, to form the respective diacetate (3) (0.55 g, 83%). Recrystallization from MeOH afforded white crystals, mp 177-178°C. *IR* (KBr, cm⁻¹): 3210, 3160, 3120, 3005, 2990, 2940, 2440, 2280, 1735, 1570, 1540, 1530, 1480, 1440, 1400, 1360, 1275, 1190,

1140, 1090, 1020, 1005, 965, 930, 905, 880, 790. *MS (EI)* m/z (relative intensity) : 343 $\{M^+, 30\}$, 301 $\{M-[CH_2=CO], 45\}$, 283 $\{M-CH_3COOH, 30\}$, 258 $\{M-[CH_3COOH]_2, 40\}$, 242 $\{[C_{12}H_{12}N_5O]^+, 90\}$, 241 $\{[C_{12}H_{11}N_5O]^+, 98\}$, 207 $\{M-136^+, 50\}$, 201 $\{30\}$, 172 $\{45\}$, 147 $\{[C_8H_7N_2O]^+, 50\}$, 97 $\{80\}$, 96 $\{[C_4H_6N_3]^+, 100\}$. *Anal.* Calcd for $C_{16}H_{17}N_5O_4$: C, 55.97; H, 4.95; N, 20.41. Found: C, 55.53; H, 5.03; N, 20.11.

1-(*N*-Methylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine Iodide (4). The title compound was prepared by exposing a solution of 1-pyridoxylidene-2-(2'-pyrimidyl)hydrazine (**1**) (1 g, 4 mmol) in 50 mL of dry ethanol to the action of methyl iodide (0.6 mL, 10 mmol) for 24 h, at refluxing temperature. The unreacted starting material was removed by filtration, the filtrate was concentrated to a volume of 10 mL, cooled, and the emerging mustard-yellow solid product was recrystallized from MeOH, to yield crystals (0.4 g, 25%), mp 252-253°C. The product appeared to be polyformic, transforming spontaneously into a distinctly different crystalline form, mp 269-270°C. *MS (EI)* m/z (relative intensity) : 259 $\{M^+-CH_3I, 50\}$, 165 $\{[C_8H_7N_2O_2]^+, 80\}$, 150 $\{[C_8H_6NO_2]^+, 95\}$, 128 $\{[HI]^+, \text{base peak } 100\}$, 110 $\{[C_6H_6NO]^+, 98\}$, 68 $\{[C_3H_4N_2]^+, 80\}$. *Anal.* Calcd for $C_{13}H_{16}N_5O_2 \cdot I \cdot H_2O$: C, 37.23; H, 4.30; N, 16.70; I, 30.31. Found: C, 37.68; H, 4.11; N, 16.51; I, 31.50.

1-(*N*-Ethoxycarbonylmethylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine bromide (5a). 1-Pyridoxylidene-2-(2'-pyrimidyl)hydrazine (**1**) (10.4 g, 40 mmol) was mixed with ethyl bromoacetate (20 g, 0.12 mol) and dry ethanol (700 mL) and allowed to reflux with stirring for 48 h. The condensation product (16.2 g, 95%) crystallized at the end of the reaction. Recrystallization from dry ethanol yielded yellow crystals, mp 188°C. *IR* (KBr, cm^{-1}) : 3280, 3150, 2960, 2860, 2820, 1740, 1638, 1565, 1530, 1440, 1405, 1370, 1322, 1286, 1255, 1220, 1170, 1088, 1060, 1024, 982, 962, 915, 850, 800, 744, 722, 670, 620. *UV* (MeOH, c. 5×10^{-6} M) λ_{max} 383.1 nm (log ϵ 4.25), 379.6 (4.24), 239.4 (4.12), 214.6 (4.05), 205.1 (4.07). 1H NMR (300 MHz, DMSO- d_6) : δ 14.12 (1H, s), 12.61 (1H, s), 8.67 (2H, d, $J = 0.8$ Hz), 8.57 (1H, s), 8.51 (1H, s), 7.33 (1H, t, $J = 1.6$ Hz), 6.00 (1H, m), 5.72 (2H, s), 4.79 (2H, s), 4.26 (2H, q, $J = 2.3$ Hz), 2.62 (3H, s, CH_3), 1.26 (3H, t, $J = 1.9$ Hz, CH_3). ^{13}C NMR (300 MHz, DMSO- d_6) : δ 169.9, 161.89, 160.39, 154.45, 149.05, 139.78, 137.58, 136.69, 131.72, 118.61, 67.47, 61.30, 16.20, 15.61. *MS (EI)* m/z : 259 $\{M^+-EtOCOCH_2Br\}$, 165 $\{[C_8H_7N_2O_2]^+, 150\}$, 123, 110 $\{[C_4H_6N_4]^+, \text{base-peak}, 100\}$, 108 $\{[C_4H_4N_4]^+, \text{base-peak}, 100\}$. *Anal.* Calcd for $C_{16}H_{22}N_5O_4Br$: C, 44.87; H, 5.18; N, 16.35; Br, 18.02. Found: C, 43.49; H, 4.68; N, 15.83; Br, 17.80.

Conversion of 5a into the Corresponding Perchlorate (5b). When the white crystals (1.0 g, 2.4 mmol) of **5a** were added to a 5% $HClO_4$ solution (7.5 mL) at rt, immediate dissolution was observed. On short standing, a yellow perchlorate crystallized out (mp 126°C). Recrystallization from methanol yielded bright yellow crystals of 1-(*N*-ethoxycarbonylmethylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine perchlorate, melting at 201-202°C (**5b**). *FTIR* (KBr, cm^{-1}) : 4370, 3300, 3212, 3094, 3045, 3008, 2981, 2943, 2906, 2845, 2732, 2654, 2363, 2341, 1702, 1654, 1649, 1635, 1507, 1453, 1417, 1378, 1334, 1292, 1278, 1265, 1176, 1039, 1016, 993, 929, 877, 854, 798, 745, 728, 689, 679, 669, 645, 623, 609, 597, 583, 566, 523, 420, 407. *UV* (MeOH, c. 1×10^{-3} M) λ_{max} 611.8 nm (log ϵ 1.85), 384.8 (4.35), 379.3 (4.36), 343 (4.08), 339.3 (4.08), 286.4 (3.69), 240.6 (4.24), 215.5 (4.20). 1H NMR (300 MHz, DMSO- d_6) : δ 13.7 (1H, m), 12.60 (1H, s), 8.65 (2H, d, $J = 0.8$ Hz), 8.52 (1H, s), 8.47 (1H, s), 7.11 (1H, t, $J = 1.58$ Hz), 5.90 (1H, m), 5.69 (2H, s), 4.78 (2H, s), 4.25 (2H, dd, $J = 1.2$, $J = 1.1$ Hz), 3.44 - 3.28 (4H, six peaks), 2.60 (3H, s), 1.26 (3H, t, $J = 2.3$ Hz). ^{13}C NMR (300 Mz, DMSO- d_6) : δ 165.65; 158.71; 158.13; 152.56; 144.41; 135.54; 135.27; 134.46; 128.25; 115.36; 62.26; 58.00; 57.90; 13.59; 12.52. *MS (EI)* m/z : 254 $\{M^+-[ClO_4]-[C_4H_3N_3]+H, 2\}$, 146 $\{[C_8H_6N_2O]^+, 47\}$, 119 $\{[C_7H_5NO]^+,$

25}, 110 {[C₄H₆N₄]⁺, 78}, 94 {[C₄H₄N₃]⁺, base peak, 100%}, 81 {[C₄H₅N₂]⁺, 66}, 68 {[C₃H₄N₂]⁺, 64}. *Anal.* Calcd for C₁₆H₂₂N₅O₈Cl : C, 42.91; H, 4.95; N, 15.64; Cl, 7.65. Found : C, 41.42; H, 4.74; N, 14.57; Cl, 8.61.

Crystal Data. Compound (**5b**), mp 202°C, space group P2₁/c, [C₁₆H₂₀N₅O₄]⁺ ClO₄⁻, M_r = 445.8, a = 12.363(3), b = 17.168(5), c = 9.657(3) Å, V = 2044.4(9) Å³, Z = 4, ρ_{calcd}, g cm⁻³ = 1.45, μ(MoKα), mm⁻¹ = 1.94, no. of unique reflections = 2568, no. of reflections with I ≥ 2σ(I) = 1196, and no. of parameters = 152. R = 0.090, R_w = 0.100, w⁻¹ = Δ²_F + 0.000629F², unit weights were used.

X-Ray Crystal Structure Analysis. Data (see Table 1) were recorded on a PW1100 / 20 Philips Four-Circle Computer-controlled Diffractometer. MoKα (λ=0.71069 Å) radiation with a graphite crystal monochromator in the incident beam was used. Unit cell dimensions were obtained by a least-square fit of 24 centered reflections in the range of 11° ≤ Θ ≤ 14°. Intensity data were collected using the ω - 2Θ technique to a maximum 2Θ of 45°. The scan width, Dw, for each reflection was 1.00 + 0.35 tanΘ with a scan speed of 3.0 deg/min. Background measurements were made for a total of 20 seconds at both limits of each scan. These standard reflections were monitored every 60 min. No systematic variations in intensities were found. Intensities were corrected for Lorentz and polarization effects. All the non-hydrogen atoms were found by using the results of the SHELX-86 direct method program.^{19a} After several cycles of refinement^{19b} the positions of the hydrogen atoms were found and added with a constant isotropic temperature factor of 0.08 Å²^{19a} to the refinement process. Convergence was obtained by minimizing the function Σω(|F_o| - |F_c|)². A final difference Fourier synthesis map revealed several peaks of less than 0.4 e/Å³ scattered about the unit cell without any significant features. The discrepancy indices, R = Σ||F_o| - |F_c|| / Σ|F_o| and R_w = {Σω(|F_o| - |F_c|)² / Σω(|F_o|)²}^{1/2} are presented above with other pertinent crystallographic data. The equivalent isotropic B's were calculated from the equation B_{eq} = (8/3)π²(u₁₁ + u₂₂ + u₃₃). Only 5 atoms of perchlorate atoms were refined anisotropically, all others none-hydrogen atoms were refined isotropically, while all hydrogens were riding on their atoms. Tables of atomic coordinates and the isotropic B equivalents of the thermal parameters are presented in Table 1. Bond distances and bond angles in Table 2. Additional data are available from the author (S.S.).

RESULTS AND DISCUSSION

The reaction of pyridoxal hydrochloride with 2-hydrazinopyrimidine (see Scheme 2) affords a high yield of the pure hydrochloride salt of 1-pyridoxylene-2-(2'-pyrimidyl)hydrazine (**1a**). Crystals of the free base (**1**) suitable for single crystal analysis are obtained upon exposure of **1a** to the action of aqueous ammonia. The two hydroxyl functions of **1** can be successively acetylated by the acetic anhydride/dry pyridine method to yield first the O²-monoacetate (**2**), and then the O^{1,2}-diacetate (**3**). Either mono- or di-acetylation of **1** confer various degrees of stability on the chelator, under electron impact (EI) (see below). The pyridoxyl ring-nitrogen (N¹) is amenable to alkylation, either by methyl iodide (**1** → **4**, moderate yield), or by bromoacetate (**1** → **5a**, essentially in quantitative yield). However, under similar reaction conditions the less basic pyrimidinic ring-nitrogens are apparently *not* amenable to alkylation, and do not form the respective di-quaternary salt. Thus, of the seven heteroatoms (N^{1,2}, and O^{1,2}) of **5a**, only five (N¹, N², N⁵, O¹ and O²) actually lend themselves to chemical bonding. These include: O²-mono-, and O^{1,2}-di-acetylation (**1** → **2** → **3** N¹-protonation (**1** → **1a**), N¹-alkylation (**1** → **4**, **1** → **5a**), N₂O-Fe(III)-coordination (**1** → **6**; **1** → {**7** + **8**}; **5** → **9** → {**10** + **11**}), and formation of H⁺-contacts (O¹-H⁺...N²; O²-H⁺...N⁵).

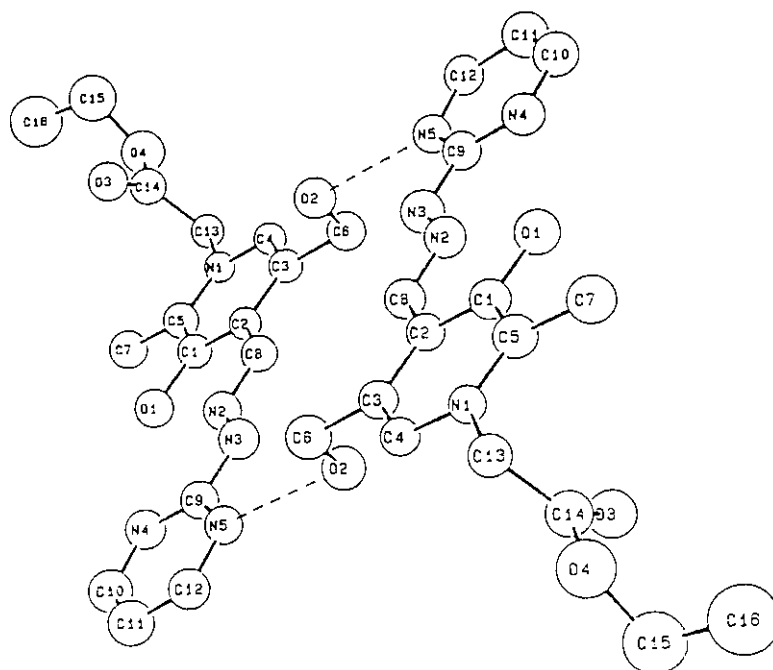


Figure 1. ORTEP drawing of 5b with the atom-numbering scheme and thermal ellipsoids at 50% probability.

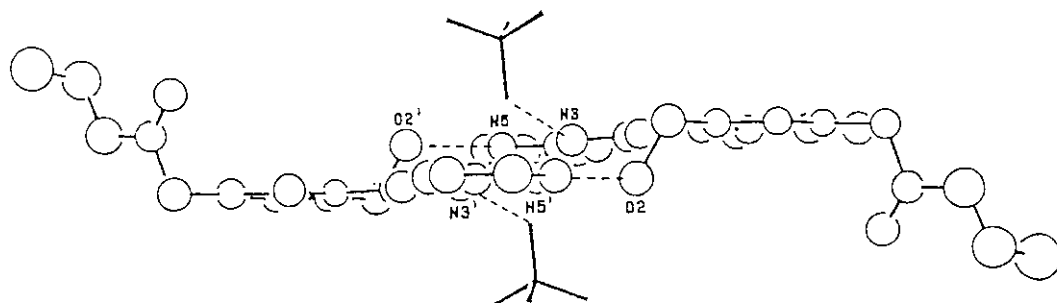


Figure 2. Perspective view of 5b.

Patterns of Mass Spectral fragmentation. For comparative study, we examined the structural effects of substitution on MS spectral fragmentation under electron impact (EI) of : i) an unprotected species, the parent chelator (1), ii) two protected species : the mono-, and the di-acetate (2 and 3), iii) the N^1 -alkylated salts of the parent chelator (4 and 5a), and iv) the perchlorate salt (5b). We found that 1, 4, and 5a exhibit similar fragmentation patterns. The N^1 -alkylated salts : 4, and 5a begin to lose the neutral RX molecule (N^1 -dealkylation), yielding the positively charged parent molecule (M^+). They then lend themselves to fission of the weak nitrogen-nitrogen bond to form ionic fragments m/z 165 and 94, followed by loss

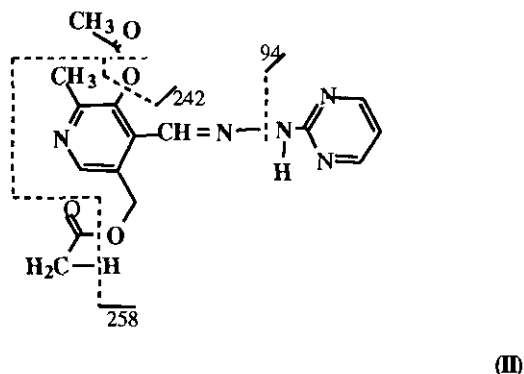
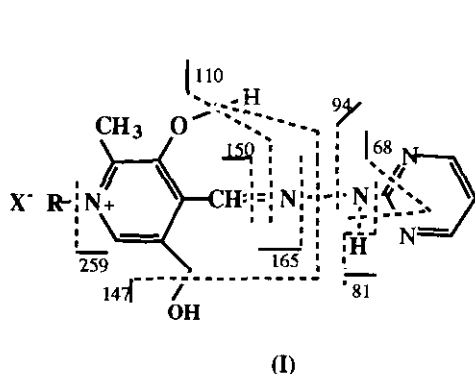
Table 1. *Positional Parameters and Isotropic Equivalent B Parameters of 1-(N-Ethoxycarbonylmethylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine Perchlorate (5b). (with Estimated Standard Deviations in Parentheses)*

Atom	X	Y	Z	B
O(1)	.1553 (6)	.0041 (4)	.5802 (9)	4.26(2)
O(2)	.4622 (6)	.1741 (4)	.8402 (9)	4.58 (3)
O(3)	.0832 (6)	.2870 (4)	.5265 (9)	4.58 (3)
O(4)	.0994 (7)	.3599 (6)	.336 (1)	6.32 (3)
N(1)	.2398 (7)	.1859 (5)	.4480 (9)	3.00 (3)
N(2)	.2993 (7)	-.0366 (6)	.770 (1)	3.95 (3)
N(3)	.3403 (8)	-.0867 (6)	.872 (1)	4.34 (3)
N(4)	.1923 (8)	-.1705 (6)	.833 (1)	4.11 (3)
N(5)	.3403 (7)	-.1977 (5)	.999 (1)	3.71 (3)
C(1)	.2174 (9)	.0682 (7)	.564 (1)	3.24 (3)
C(2)	.3149 (8)	.0786 (7)	.645 (1)	3.08 (3)
C(3)	.3753 (9)	.1458 (7)	.620 (1)	3.40 (3)
C(4)	.3357 (9)	.1986 (6)	.523 (1)	3.16 (3)
C(5)	.1791 (9)	.1231 (7)	.468 (1)	3.47 (3)
C(6)	.4837 (9)	.1619 (7)	.698 (1)	4.03 (4)
C(7)	.074 (1)	.1115 (7)	.380 (1)	4.66 (4)
C(8)	.354 (1)	.0237 (7)	.750 (1)	3.63 (3)
C(9)	.2860 (9)	-.1542 (7)	.902 (1)	3.55 (3)
C(10)	.146 (1)	-.2383 (7)	.866 (1)	4.66 (4)
C(11)	.197 (1)	-.2863 (8)	.966 (1)	5.13 (4)
C(12)	.2950 (9)	-.2646 (7)	1.029 (1)	4.34 (4)
C(13)	.2033 (8)	.2497 (7)	.354 (1)	3.47 (3)
C(14)	.1215 (9)	.2987 (7)	.419 (1)	4.03 (4)
C(15)	.029 (1)	.4206 (9)	.387 (2)	7.42 (4)
C(16)	-.079 (1)	.415 (1)	.329 (2)	8.37 (6)
Cl	.3578 (4)	.0724 (3)	.1748 (5)	6.02 (3)
O(11)	.4233 (8)	.0356 (6)	.083 (1)	7.26 (5)
O(12)	.0413 (1)	.0140 (1)	.222 (2)	15.00 (5)
O(13)	.340 (1)	.0261 (9)	.283 (2)	15.00 (5)
O(14)	.261 (1)	.0974 (1)	.117 (2)	15.26 (2)

Table 2. Bond Distances ($\text{\AA}$) and Bond Angles ($^\circ$) in 1-(*N*-Ethoxycarbonylmethylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine Perchlorate (5b) (with Estimated Standard Deviations Parantheses)

Bond Length	$\text{\AA}$	Bond Length	$\text{\AA}$
O(1)-C(1)	1.36 (1)	N(5)-C(9)	1.34 (1)
O(2)-C(6)	1.43 (1)	N(5)-C(12)	1.32 (2)
O(3)-C(14)	1.19 (1)	C(1)-C(2)	1.40 (1)
C(1)-C(5)	1.38 (2)	O(4)-C(14)	1.34 (2)
O(4)-C(15)	1.47 (1)	C(2)-C(3)	1.40 (2)
N(1)-C(4)	1.36 (1)	C(2)-C(8)	1.45 (2)
N(1)-C(5)	1.33 (1)	C(3)-C(4)	1.37 (2)
N(1)-C(13)	1.48 (1)	C(3)-C(6)	1.52 (2)
N(2)-N(3)	1.36 (1)	C(5)-C(7)	1.51 (2)
N(2)-C(8)	1.29 (2)	C(10)-C(11)	1.38 (2)
N(3)-C(9)	1.38 (2)	C(11)-C(12)	1.37 (2)
N(4)-C(9)	1.32 (1)	C(13)-C(14)	1.49 (2)
N(4)-C(10)	1.34 (2)	Cl - O(11)	1.39 (1)
Angles	$^\circ$	Angles	$^\circ$
C(14)-O(4)-C(15)	117 (1)	N(1)-C(5)-C(1)	119 (1)
C(4)-N(1)-C(5)	122 (1)	N(1)-C(5)-C(7)	120 (1)
C(4)-N(1)-C(13)	115.2 (9)	C(1)-C(5)-C(7)	122 (1)
C(5)-N(1)-C(13)	122.6 (9)	O(2)-C(6)-C(3)	106.7 (1)
N(3)-N(2)-C(8)	116 (1)	N(2)-C(8)-C(2)	119 (1)
N(2)-N(3)-C(9)	120 (1)	N(3)-C(9)-N(4)	120 (1)
C(9)-N(4)-C(10)	116 (1)	N(3)-C(9)-N(5)	113 (1)
C(9)-N(5)-C(12)	116 (1)	N(4)-C(9)-N(5)	128 (1)
O(1)-C(1)-C(2)	121 (1)	N(4)-C(10)-C(11)	120 (1)
O(1)-C(1)-C(5)	118 (1)	C(10)-C(11)-C(12)	119 (1)
C(2)-C(1)-C(5)	122 (1)	N(5)-C(12)-C(11)	121 (1)
C(1)-C(2)-C(3)	117 (1)	N(1)-C(13)-C(14)	110 (1)
C(1)-C(2)-C(8)	123 (1)	O(3)-C(14)-O(4)	125 (1)
C(3)-C(2)-C(8)	120 (1)	O(3)-C(14)-C(13)	127 (1)
C(2)-C(3)-C(4)	120 (1)	O(4)-C(14)-C(13)	108 (1)
C(2)-C(3)-C(6)	122 (1)	O(4)-C(15)-C(16)	112 (1)
C(4)-C(3)-C(6)	119 (1)	O(11)-Cl - O(12)	106.8 (8)
N(1)-C(4)-C(3)	121 (1)	O(11)-Cl - O(13)	111.2 (8)

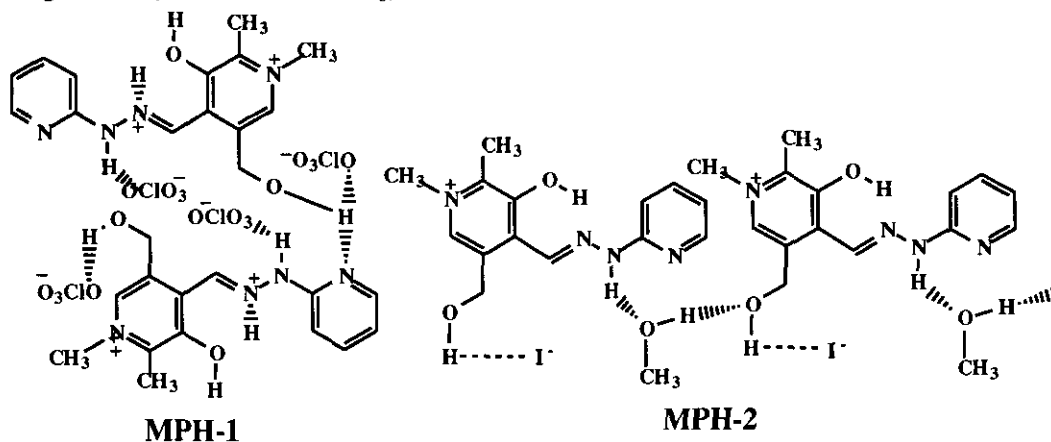
of N and formation of ionic fragments m/z 110, 108 (after loss of 2H), 94, and 68, as indicated in formula (I). The partially protected mono- O^2 -acetate (2), begins to lose its CH_3CO group, affording a molecular-ion peak (M^+) as a base-peak in the mass spectrum, then loses the OH, and finally undergoes N-N bond-fission to yield the ionic fragment m/z 94 (base-peak), attributed to amino-pyrimidine, as indicated in formula (II). Qualitatively, the fully protected diacetate (3) exhibits under EI an identical cracking pattern, as delineated by formula (II). It is worthy of note that $\text{M}^+ \rightarrow [165]^+ \rightarrow [150]^+$ mass fragmentation, shown above to be *prominent* in the unprotected species of 1, 4, and 5a, appears to be *unimportant* when the hydroxyl groups in 1 are protected. As in 2 - 3, the *prominent* peak in the EI spectrum of the perchlorate (5b) occurs at m/z 94. But, in contrast to 2 - 3, the peaks at m/z 146 (147 - H), 110, and 68, are of *importance*.



Single-Crystal Structure Analysis. From the crystallographic data assembled in Tables 1 - 4, and from the *ORTEP* drawing (Figure 1) and the *perspective* view (Figure 2) of 5b, it can be seen that in the crystalline state the molecule exists in its *dipolar form*. The observed bond-angle of 122° for the pyridoxal-ring C(4)-N(1)-C(5) angle (see Table 2), is indeed consistent with a *dipolar*, rather than with a *nondipolar form*, as the respective bond-angle for the latter should have been smaller by 5° , namely, about 117° .²⁰ Actually, the dimer is a planar dicationic 20-membered cyclic dimer {preferred motif²¹ of $\text{R}^2(20)$ }, which is stabilized by *intermolecular* $\text{O}^2\text{-H} \cdots \text{N}^5$ H-bonding. The polycyclic structure of the dimer is further stabilized by inclusion of a flat inner 6-membered ring, arising from *intramolecular* H-contacts (1.670 Å) between phenolic hydrogen {O(1)-H} and the hydrazonic sp^2 nitrogen {N(2)}. Additional stabilization is gained through the energetically-favored *anti-parallel* dispositions between the hydroxymethyl{C⁷-O²-H}, and the azinic{C(8)-N(2)} side-chain on the pyridoxal ring (see Figure 2). Furthermore, the azinic C(8)-N(2) double-bond also prefers the energetically-favored *trans* geometry. Last, but not least, is the formation of $\text{O}^2\text{-H} \cdots \text{N}^5$ contacts, affording the polycyclic dimer a stable flattened shape, as depicted in Figure 2.

Examination of the *ORTEP* drawings of 5b (Figure 2), and MPH-1²¹ discloses some major differences in their molecular dispositions, and in the geometric patterns and lengths of H-bonding. The reactions of 1-*N*-methylpyridoxylidenium-2-(2'-pyridyl)hydrazine iodide (MPH), and its analog 1-(*N*-ethoxycarbonylmethylpyridoxylidenium)-2-(2'-pyrimidyl)hydrazine bromide (5a), with 5% HClO_4 , form dimers of different character. The pyridine-containing ligand, was found to contain a bent-shaped non-cyclic dimeric di-cation, forming a 37.98° dihedral angle between the two heteroaromatic ring plane in which the di-cation framework accommodates two counter-ions by use of $(\text{N}^3)\text{-H} \cdots \text{OCIO}_3$ contacts. The open-chain dimer of PPH-1 is surprisingly stable, applying one, rather than two, *weak, non-cyclic*,

(O²⁻)-H⁺ N⁴⁺ (1.84 Å) H-bonding.^{2a} Unlike PPH-1, the di-hedral angle between the two heteroaromatic ring planes in the flattened dicationic macrocycle of **5b** is very small indeed - 1.52°. A *perspective* view of **5b** (Fig. 2) shows that the di-cationic macrocycle is virtually intercalated between two negatively charged, tetrahydral ions of {⁻OCIO₃}.



held together *via* (N³)-H⁺ ···OCIO₃ contacts. Although there is some resemblance in macrocyclic structure between the electrically neutral-*non-dipolar* form of PIH and the electrically neutral-*non-dipolar* form of PIH and the *dipolar* (ionic) form of **5b**, they differ in the electric charges on their dimeric rings. This highlights the predominant importance of steric factors in the process of molecular assembly. Unlike the *dipolar* (**5b**), which utilizes the strongest character (2C) of H-bonding (O²⁻-H⁺ N⁴⁺) for assembling its 20-membered ring dimer, the *non-dipolar* PIH conversely applies the weakest (1.850 Å) type²² of the *non-linear three-centered* (3C) sort of H-bonding (N³-H⁺ O²⁻) in forming its *endo-cyclic* 16-membered-ring dimer. However, to form the *exo-cyclic* H-contacts required in the assembly of its tetramer, PIH uses the strongest kind (1.726 Å) of H-bonding,²³ that of "*linear two-center*" (2C) nature. Although the tendency to assemble in dimers is shared by both PIH and MPH, they differ in their tendency to affect the ring-closure of the *non-cyclic* dimer, affording large rings. Thus, the tendency to form cyclic-dimers appears to decrease considerably on going from PIH, to MPH-1, to MPH-2, most likely due to polar effects. This highlights the complexity in the interplay between the polar and steric effects in this class of chelators, arising from the aromatic nature of the heteroannular ring-systems, substitution, and protonation.²⁴ Admittedly, this facet deserves more thorough studies.

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REFERENCES

- 1 Part 6. S. Sarel, S. Cohen, and S. Avramovici-Grisaru, *Heterocycles*, **1998**, *47*, 1033.
- 2 (a) S. Avramovici-Grisaru, S. Cohen, and S. Sarel, *Heterocycles*, **1990**, *30*, 1079; (b) S. Avramovici-Grisaru, S. Cohen, and S. Sarel, *J. Org. Chem.*, **1990**, *55*, 5236.
- 3 P. Doungdee, S. Sarel, N. Wongvisetsirikul, and S. Avramovici-Grisaru, *J. Chem. Soc., Perkin Trans. 2*, **1995**, 319.
- 4 (a) S. Sarel, S. Avramovici-Grisaru, C. Hershko, G. Link, and D. Spira, *European Patent Application*,

- 0 315 434 A2, 10 May 1989 (*Chem. Abstr.*, 1990, 112, P 98388^m); (b) C. Hershko, S. Avramovici-Grisaru, G. Link, L. Gelfand, and S. Sarel, *J. Lab. Clin. Med.*, **1981**, 98, 99; (c) G. M. Brittenham, *Annal N.Y. Academy of Sciences*, **1990**, 612, pp. 315-326.
- 5 S. Avramovici-Grisaru, S. Sarel, G. Link, and C. Hershko, *J. Med. Chem.*, **1983**, 26, 298.
- 6 P. Ponka, J. Borova, J. Neuwirt, O. Fuchs, and E. Necas, *Biochim. Biophys. Acta*, **1979**, 586, 278.
- 7 (a) E. N. Iheanacho, S. Sarel, A. Samuni, S. Avramovici-Grisaru, and D. T. Spira, *Trans. R. S. Trop. Med. Hyg.*, **1990**, 84, 213; (b) E. N. Iheanacho, S. Sarel, A. Samuni, S. Avramovici-Grisaru, and D. T. Spira, *Free Rad. Res. Commun.*, **1991**, 11, 307 (c) E. N. Iheanacho, S. Sarel, A. Samuni, S. Avramovici-Grisaru, and D. T. Spira, *Free Rad. Res. Commun.*, **1991**, 15, 1.
- 8 (a) S. Sarel, C. Fizames, F. Lavell, and S. Avramovici-Grisaru, *J. Med. Chem.*, *in press*; (b) Abst. of papers, 3rd NIH sponsored symposium on *The Development of Iron Chelators for Clinical Use*, Gainesville, Florida, May 20-22, **1992**, pp. 46-47.
- 9 V. P. Namaev, O. P. Shkurko, and S. G. Baram, *Adv. Heterocyclic Chem.*, **1987**, 42, 1.
- 10 S. Avramovici-Grisaru, S. Cohen, S. Sarel, and R. E. Bauminger, *Isr. J. Chem.*, **1985**, 25, 288.
- 11 J. C. Vederas and H. G. Floss, *Acc. Chem. Res.*, **1980**, 13, 455.
- 12 P. Doungeedee, S. Sarel, I. Ringel, D. Gibson, N. Wongwisetsirikul, and S. Avramovici-Grisaru, *Heterocycles*, **1995**, 40, 241.
- 13 (a) S. Sarel, S. Avramovici-Grisaru, and S. Cohen, *J. Chem. Soc., Chem. Commun.*, **1986**, 47; (b) L. A. Summers, *Adv. Heterocyclic Chem.* **1984**, 35, 350; (c) P. Hanson, *Adv. Heterocyclic Chem.*, **1979**, 25, 205; *Adv. Heterocyclic Chem.*, **1980**, 27, 32; (d) C. L. Bird and A. T. Kuhn, *Chem. Soc. Rev.*, **1981**, 10, 49.
- 14 The fate of the ⁵⁹Fe tracer in normal rats injected with iron chelators was shown that the total excretion of radioiron increases from 26.4±1.3 for PIH, to 39.2±1.6 (dime-PIH), to 86.7±1.7 (EPP), which tallies with the 85.4±4.3 found for desferrioxamine B.¹⁶
- 15 The only chelator in current clinical use for removal of iron surpluses in transfusional iron overload.^{4c}
- 16 D. J. Brown, *The Pyrimidines*, Interscience Publishers, **1962**, London.
- 17 J. Brown and P. W. Ford, *J. Chem. Soc.*, **1967** (C), 568, give for 2-hydrazinopyrimidine, mp 112-113°C, λ_{max}(EtOH) 230 nm (log ε 4.13), 300 nm (3.38); pK_a = 4.55±0.04.
- 18 G. M. Sheldrick, *Crystallographic Computing*, 3, Oxford University Press, **1985**, pp. 175-189.
- 19 (a) C. L. MacLaurin and M. F. Richardson, *Acta Cryst.*, **1985**, C41, 261. (b) All crystallographic computing was done on a CYBER 855 computer at the Hebrew University of Jerusalem, using SHELX 1977 Structure Determination Package.
- 20 (a) M. C. Etter, *Acc. Chem. Res.*, **1990**, 23, 120; (b) M. C. Etter and D. A. Adsmond, *J. Chem. Soc., Chem. Comm.*, **1990**, 589.
- 21 (a) G. A. Jeffrey and H. Maluszynska, *Int. J. Biol. Macromol.*, **1982**, 4, 173; (b) R. Taylor and O. Kennard, *Acc. Chem. Res.*, **1984**, 17, 320; (c) G. A. Jeffrey and J. Mitra, *J. Amer. Chem. Soc.*, **1984**, 106, 5546; (d) R. Taylor, O. Kennard, and W. Versichel, *J. Amer. Chem. Soc.*, **1984**, 106, 244.
- 22 (a) J. E. DelDene and J. A. Pople, *J. Phys. Chem.*, **1972**, 52, 4858; (b) W. C. Hamilton and J. A. Ibers, *Hydrogen Bonding in Solids*, W. Benjamin Inc. New York, **1968**; (c) J. C. Speakman, *Structure Bonding*, **1972**, 12, 148; (d) A. Novak, *Structure Bonding*, **1974**, 18, 77.
- 23 R. Gallo, C. Roussel, and U. Berg, *Adv. Heterocyclic Chem.*, **1988**, 43, 173.

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